

Specification

A. Specification paragraph:

The paragraphs on pages 4, 11, 39, and 40 of the specification were amended to provide clarification and to include the appropriate SEQ ID NO, in compliance with 37 C.F.R. § 1.821-1.825. These amendments have merely changed the SEQ ID NOs used to identify the disclosed sequences in order to remove redundant SEQ ID NOs and to conform with the amended Tables and Figures. These amendments do not substantively alter the disclosed subject matter and do not constitute new matter. A copy of the marked up original paragraphs are attached as **Appendix B**.

B. The Amended Tables:

The Tables VII-VIII (pages 53-55) of the specification were amended to re-number the SEQ ID NOs for the sequences included therein, and to remove two redundant sequences in Table VII, so that the numbers conform with numbering found in the sequence listing, submitted herewith. Additionally, each sequence disclosed in Table VIII is now identified by a unique SEQ ID NO, in compliance with CFR §§ 1.821-1.825. These amendments do not constitute new matter, and only serve to clarify the invention and bring the application into compliance with the sequence listing rules. New tables VII-VIII are found on substitute sheets pages 53-55, submitted herewith.

No new matter has been added by way of any of these amendments to the specification.

Figures:

Revised Figures 1-7 were amended to include the appropriate SEQ ID NOs in order to identify each disclosed sequence in compliance with 37 C.F.R. § 1.821-1.825. These amendments have been made to clarify further the subject matter of the Figures. No new matter has been added by way of these amendments. A marked-up version of the originally filed Figures 1-7 showing changes made by these amendments are attached as **Appendix C**.

Sequence Listing:

In compliance with 37 C.F.R §§ 1.821-1.825, the applicant herewith submits the Sequence Listing on paper under 37 C.F.R. § 1.821(c), and computer readable form copy (CRF) on 3.5" disk under 37 C.F.R. § 1.821(e). The content of the paper copy and the computer readable form of the Sequence Listing are the same.

The Sequence Listing has been generated from the specification and does not constitute new subject matter.

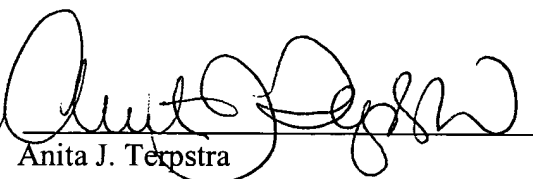
Conclusion

The Commissioner is hereby authorized to charge payment of any fees required in connection with the papers transmitted herewith, or to credit any overpayment of same, to Deposit Account No. 13-2490.

If the Examiner has any questions regarding this Response and Amendment, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,
**McDONNELL BOEHNEN HULBERT
& BERGHOFF**

Date: November 7, 2001

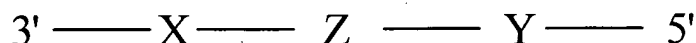
By 
Anita J. Terpstra
Registration No. 47,132

APPENDIX A
Version With Markings to Show Changes Made to Claims

Bold, underline text, **for example**, indicates inserted text
Bracketed, strikethrough text, [~~for example~~], indicates deleted text

IN THE CLAIMS:

2. (Amended) A nucleic acid molecule with endonuclease activity having the formula II:



wherein, N represents a nucleotide or a non-nucleotide linker; X and Y are independently oligonucleotides of length sufficient to stably interact with a target nucleic acid molecule; Z is an oligonucleotide having a nucleotide sequence selected from the group consisting of 5'-AGAUAACGUGAAGAU-3' (SEQ ID NO [53]**97**) and 5'-AAUGGCCUAUCGGUGCGA-3' (SEQ ID NO [54]**98**); _____ represents a chemical linkage; and C, G, A, and U represent cytidine, guanosine, adenosine and uridine nucleotides, respectively.

APPENDIX B
Version With Markings to Show Changes Made to Specification

Bold, underline text, **for example**, indicates inserted text
Bracketed, strikethrough text, [~~for example~~], indicates deleted text

IN THE SPECIFICATION:

The paragraph on page 4, line 7 – page 5, line 10:

In the above formulae, each N represents independently a nucleotide or a non-nucleotide linker, which may be same or different; X and Y are independently oligonucleotides of length sufficient to stably interact (*e.g.*, by forming hydrogen bonds with complementary nucleotides in the target) with a target nucleic acid molecule (the target can be an RNA, DNA or RNA/DNA mixed polymers, including polymers that may include base, sugar, and/or phosphate nucleotide modifications; such modifications are preferably naturally occurring modifications), preferably, the length of X and Y are independently between 3-20 nucleotides long, *e.g.*, specifically, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, and 20); X and Y may have the same lengths or may have different lengths; m, n, o, and p are integers independently greater than or equal to 1 and preferably less than about 100, specifically 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 50; wherein if (N)_m and (N)_n and/or (N)_o and (N)_p are nucleotides, (N)_m and (N)_n and/or (N)_o and (N)_p are optionally able to interact by hydrogen bond interaction; preferably, (N)_m and (N)_n and/or (N)_o and (N)_p independently form 1, 2, 3, 4, 5, 6, 7, 8, 9 base-paired stem structures; D is U, G or A; L₁ and L₂ are independently linkers, which may be the same or different and which may be present or absent (*i.e.*, the molecule is assembled from two separate molecules), but when present, are nucleotide and/or non-nucleotide linkers, which may comprise a single-stranded and/or double-stranded region; _____ represents a chemical linkage (*e.g.* a phosphate ester linkage, amide linkage or others known in the art); • represents a base-pair interaction; Z is independently a nucleotide sequence selected from the group comprising 5'-AGAUAACGUGAAGAU-3' (SEQ ID NO [53]97) and 5'-AAUGGCCUAUCGGUGCGA-3' (SEQ ID NO [54]98), additions, deletions, and substitutions to these sequences may be made without significantly altering the activity of the molecules and are hence within the scope of the invention; and C, G, A, and U represent cytidine, guanosine, adenosine and uridine nucleotides,

respectively. The nucleotides in each of the formulae I and II are unmodified or modified at the sugar, base, and/or phosphate as known in the art.

The paragraph on page 11, lines 26-28:

Figure 7 is a representative diagram of structural similarities between Class I (SEQ ID NO [446]**117**), (substrate SEQ ID NO [447]**103**) and Class VIII (SEQ ID NO [448]**121**), (substrate SEQ ID NO [449]**103**) enzymatic nucleic acid molecules of the invention.

The paragraph on page 39, lines 2-15:

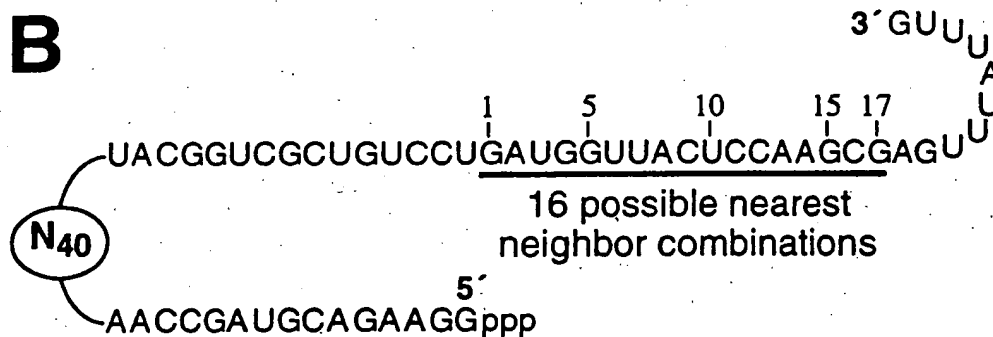
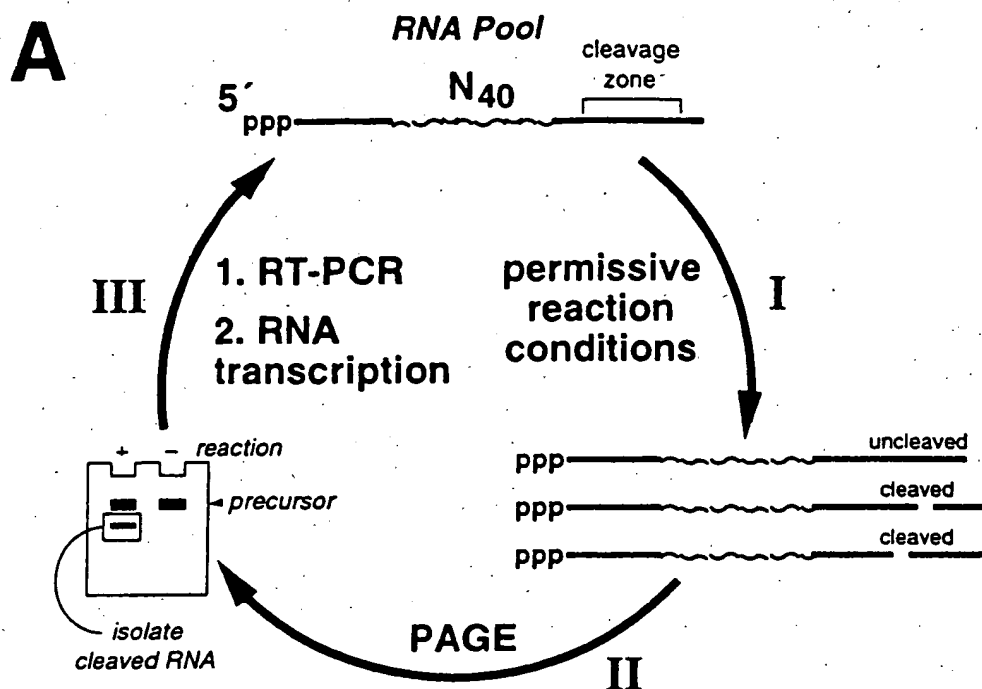
The initial population of RNA for *in vitro* selection was created by first generating a double-stranded DNA template for *in vitro* transcription. SuperScript® II reverse transcriptase (RT, GibcoBRL) was used to extend 280 pmoles of the DNA oligonucleotide "primer 1" (5'-GAAATAAACTCGCTTGGAGTAACCATCAGG-ACAGCGACCGTA-3') (SEQ ID NO [55]**99**; region representing 16 possible nearest neighbor combinations is underlined) using 270 pmoles of the template DNA (5'-TCTAATACGACTCACTATAGGAAGACGTAGCCAA-N₄₀TACGGTCGCTGTCCTG-3') (SEQ ID NO [56]**100**; T7 promoter is underlined and N represents an equal mixture of the four standard nucleotides). The extension reaction was conducted in a total of 50 µl containing 50 mM Tris-HCl (pH 8.3 at 23°C), 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol (DTT), 0.2 mM each of the four deoxyribonucleoside-5' triphosphates (dNTPs), and 10 U µl⁻¹ RT by incubation at 37°C for 1 hr. The resulting double-stranded DNA was precipitated by the addition of 5 µl 3 M sodium acetate (pH 5.5) and 140 µl 100% ethanol and pelleted by centrifugation. This extension reaction provides ~10¹⁴ different template sequences.

Please replace the paragraph on page 39, line 27 – page 40, line 10 with the following:

" The initial selection reaction (G0) contained 2000 pmoles of RNA in a total of 400 µl reaction buffer (50 mM HEPES [pH 7.5 at 23°C], 250 mM KCl and 20 mM MgCl₂) and was incubated at 23°C for 4 hr. The reaction was terminated by the addition of EDTA and the RNA was recovered by precipitation with ethanol. RNA cleavage products were separated by denaturing 10% PAGE,

visualized and quantified using a Molecular Dynamics PhosphorImager®, and the gel region corresponding to the location of the desired RNA cleavage products was excised. The RNA was recovered from the excised gel by crush-soak elution followed by precipitation with ethanol. The selected RNAs were amplified by RT-PCR as described previously (10) using primers 1 and 2 (5'-GAATTCTAATACGACTCACTATAGGAA-GACGTAGCCAA-3') (SEQ ID NO [57]101); T7 promoter is underlined). The resulting double-stranded DNA from each round of *in vitro* selection was used to transcribe the RNA population for the subsequent round, in which all steps were conducted at ~1/10th the scale of G0. All other parameters of the selection process were maintained as in G0. Representative ribozymes from the populations derived from 6, 9, 12 and 15 rounds of selection were examined by cloning (TOPO®-TA cloning kit; Invitrogen) and sequencing (ThermoSequenase® kit; Amersham Pharmacia).

Figure 1



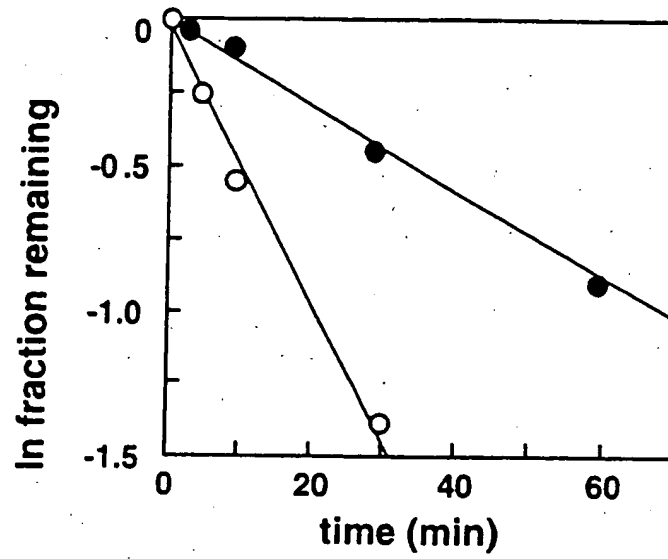
(SEQ ID NO 58)
(SEQ ID NO 102)

Figure 1 displays 16 RNA secondary structure diagrams, each representing a different RNA construct. The diagrams are arranged in a 4x4 grid. Each diagram is labeled with a class (G6, G12, G15, G16, G9, G13, G10, G11), a class number (I-IV, VII, XII, XIII, VI, X, IX), and a k_{obs} value. The diagrams show various RNA folds, including hairpins, internal loops, and complex tertiary structures. Some diagrams are labeled with 'substrate' and 'enzyme' regions. The diagrams are arranged in a grid with rows and columns labeled with class numbers and k_{obs} values.

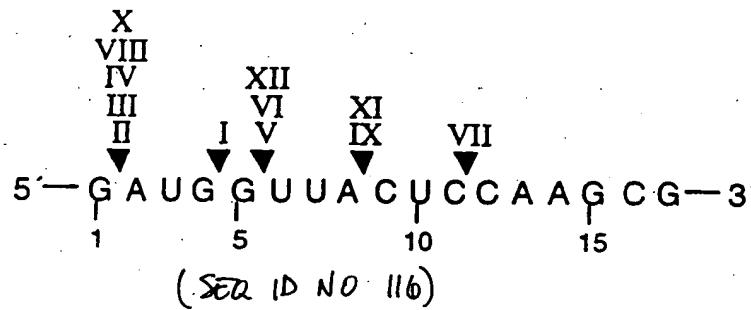
Class	Class Number	k_{obs} (min ⁻¹)	Diagram Label
G6	Class I	3×10^{-2}	103
G12	Class VII	1×10^{-1}	103
G15	Class XII	1×10^{-1}	103
G16	Class XIII	3×10^{-4}	106
G9	Class VI	2×10^{-4}	109
G13	Class X	8×10^{-4}	103
G10	Class IX	3×10^{-2}	103

Figure 3

A

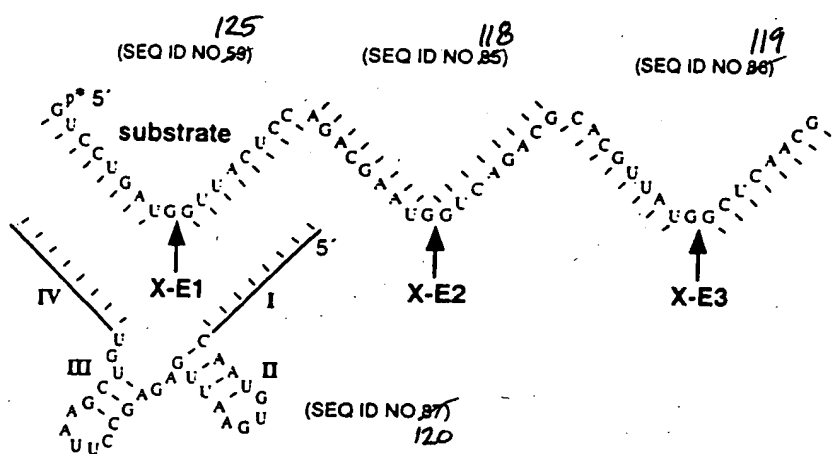


B



(SEQ ID NO 82)

Figure 5



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Figure 6: Class V ribozyme 2'-O-methyl core "walk"

Rate for 6@7 2'-O-Me arms and all ribo core $K_{obs} = 0.056$ and 0.058 min^{-1}

Rate for all 2'-O-Me enzyme with A14.1 = ribo $K_{obs} = 0.00008 \text{ min}^{-1}$

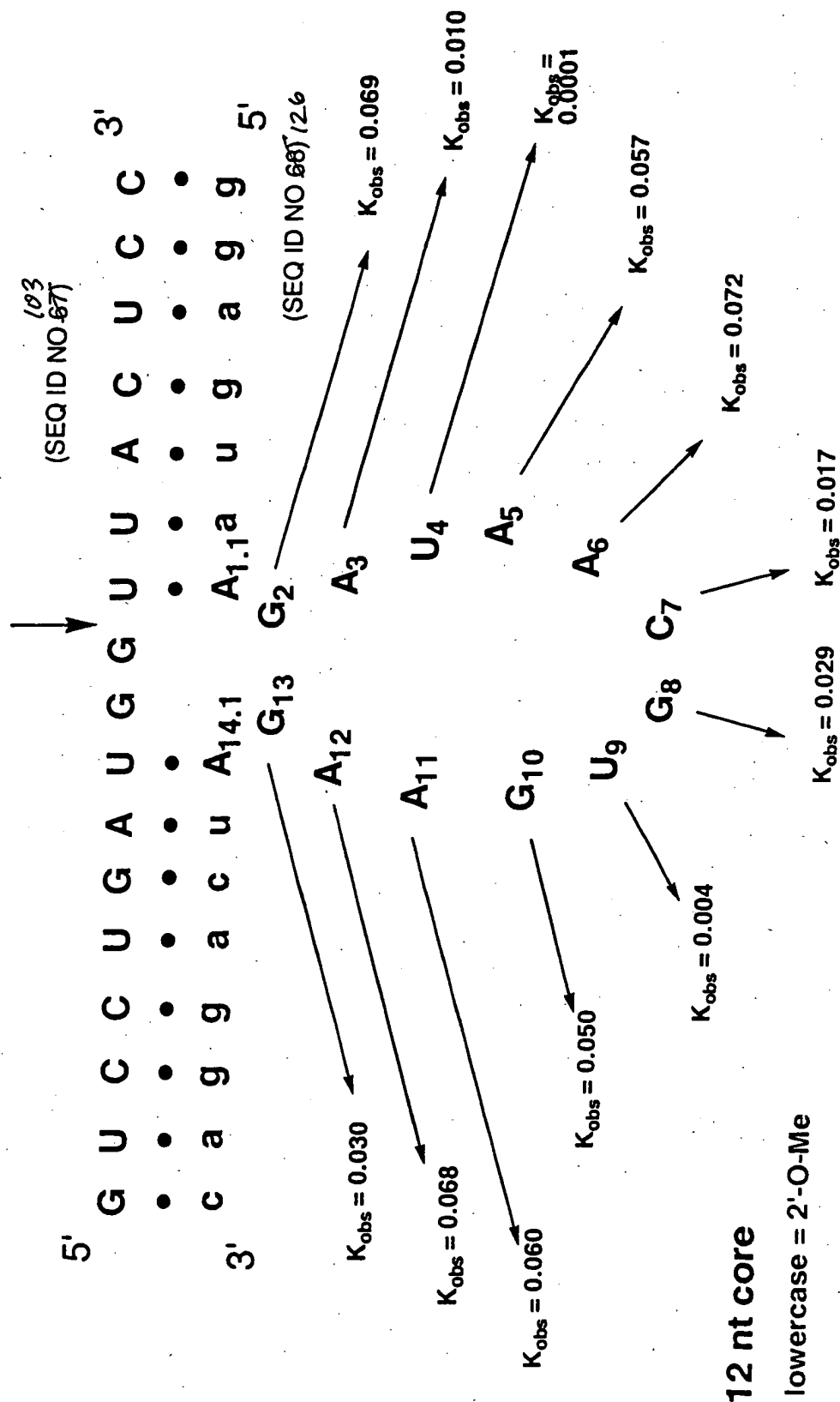
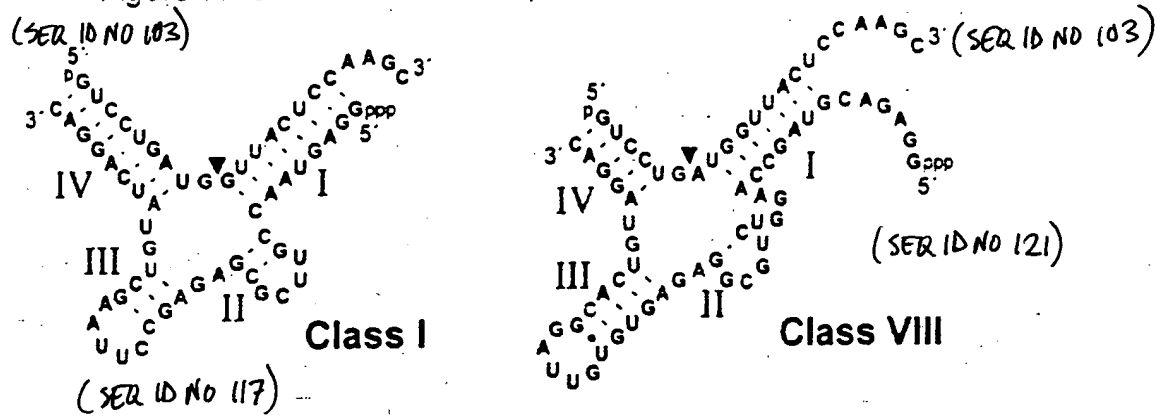
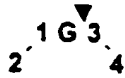


Figure 7: Class I and VIII Sequence and Structural Similarities



Class I motif cleavage site



1 = A or U

2 = complementary to 1

3 = G, A or U

4 = complementary to 3